Synopsis

Characterization and Bioevaluation of green synthesized Silver Nanoparticles using seed and *in vitro* grown tissue extract of two Indian millets: *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen

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STATE OF ART:

Various health problems and illnesses, ranging from mild infections to dreadful diseases such as cancer have continued to pose major global health concerns in recent decades and require immediate attention[1&2]. According to the report of WHO the most common cancer includes breast, lung, prostate, colon and rectum cancers. The reason behind the occurrence of cancer can be due to various reasons. For instance, genetic abnormalities account for just 5–10% of cancer instances; lifestyle and environmental factors account for 90–95% of cases. Another significant and well-known cause of many cancers is infections by different bacteria and viruses[3&4]. Major changes in lifestyle can be attributed to a significant shift in dietary habits. Unhealthy food habits have been linked to a number of illnesses such as diabetes, cancer, inflammatory diseases and obesity. The management of these illnesses primarily involves the use of synthetic medications[5]. Taking cancer into major consideration, traditional approaches for treatment include radiotherapy or chemotherapy[6]. However, the synthetic drugs posses various complications, pernicious effects and numerous drawbacks, thus the hunt for safer and novel natural compounds is a constant emphasis[5&6]. Plant systems naturally produce substances known as phytochemicals, which may have positive effects on health and can be applied as tools for treating and managing diseases[5&7].

Besides, people at present are becoming more conscious of what they are eating. The idea of food is evolving. Gone are the days when the focus was on survival, sating hunger, and the absence of harmful effects on health. Instead, people are using nutraceutial foods, which promise to improve overall health and well-being and lower the risk of chronic illnesses like obesity, some types of cancer, and cardiovascular diseases[8].

The key component of nutraceutical food which is necessary to mitigate the risk of chronic illness is antioxidants. Antioxidants are micronutrients that have garnered attention in recent years because of their capacity to counteract the effects of free radicals. Free radicals are byproducts of a variety of physiological processes including aerobic respiration and substrate oxidation and inflict serious damage on vital biomolecules such as lipids, carbohydrates, proteins and nucleic acids. Long-term failure to eliminate active oxygen molecules can result in arthritis, diabetes, cancer, gastritis, cardiovascular disease, and a variety of neurological disorders. Studies have shown that lipid peroxidation caused by the reactive oxygen species may be responsible for carcinogenesis[8,9&10].The development of cancer has been associated with the generation of reactive oxygen species[11].

Millets are a group of cereal crops belonging to the family Poaceae, that are regarded as the first cereal crop to be cultivated from the dawn of human civilization thousands of years ago[12&13]. They are cultivated worldwide for food as well as fodder and grow well in dry zones, under marginal conditions of soil fertility and moisture. They are even able to thrive in harsh environmental conditions such as drought[14&15]. Millets that were once considered poor man's food are now a common sight on the plates of well-to-do and health-conscious individuals[15]. This is because millet grains are rich in protein, dietary fibre, micronutrients, and have a low glycemic index (GI). This has ordained them with the title of 'nutricereals'[16&17]. According to studies, whole grain cereals include bioactive phytochemicals that may have positive health impacts in addition to the essential nutrients like carbohydrates, proteins, fats, minerals, and vitamins[18].

Millets are known to have high concentrations of phytochemicals, such as flavonoids, tannins, phenolics, and carotenoids, that shows potent antioxidant properties and retard the growth of tumors. Phytochemicals found in millets are worthy of attention due to their potential as nutraceuticals with antioxidant, anti-inflammatory, antidiabetic, anti-carcinogenic, antimicrobial, antiulcer, and anti-cardiovascular effects[17,18&19]. Antioxidants found in whole grains such as vitamin E, phenolic acids, tocotrienols, lignans, and phytic acid are stated to be chemopreventive in some studies[3&20].

Millets are subdivided into two groups- major (pearl millet, sorghum and finger millet) and minor millets (foxtail, kodo, proso, little, barnyard and browntop millet). The present study is based on two Indian minor millets.

Paspalum scrobiculatum L. commonly known as kodo millet is cultivated on a large basis in countries such as India, China, Japan and Africa. Its alternative names include rice grass, cow grass and ditch millet[15,16&20]. The local names vary from region to region being named as Harka in Kannada, Kodo in Bengali, Kodra in Gujarati, Punjabi and Marathi, Kodon in Hindi, Kodua in Odia, Arika in Telugu and Varagu in Tamil[15&16]. Within India, it is majorly cultivated in the Deccan Plateau including the states of Gujarat, Karnataka and Tamil Nadu, some regions of Maharashtra, Odisha, West Bengal, Uttar Pradesh and Rajasthan. Kodo millet is an excellent source of protein, and fibre, as compared to rice and wheat[15&16]. In ancient Ayurveda, it is called Langhna, which means food that provide lightness to the body. Traditionally, the grains of this millet are employed in the management of diabetes mellitus[21] which is also mentioned in Ayurvedic writings such as the Charak Samhita and Shushruta

Samhita. Studies have shown that *Paspalum scrobiculatum* L. possess significant antioxidant, antidiabetic, antibacterial and antiproliferative activity[9,13,19,22&23].

Urochloa ramosa (L.) T.Q.Nguyen earlier known as *Panicum ramosum* L. and *Brachiaria ramosa* (L.) Stapf commonly known as browntop millet is small-seeded annual grass cultivated as a grain crop, primarily on the marginal lands in dry areas in temperate, subtropical and tropical regions. In India, it is grown in states like Karnataka and Andhra Pradesh[24&25]. The local name differs by region, being named "Korale" in Kannada, "Karlakki" in Mandya, and "Andukorralu" in Telangana and Andhra Pradesh[25,26&27]. The grains of this millet are abundant in protein, carbohydrates, and dietary fibre. Apart from these basic nutrients, they are also rich sources of minerals such as iron, phosphorus, potassium, magnesium, zinc and manganese and various phytochemicals such flavonoids, quinones, tannins and resin[24]. Therefore, they are believed to provide immunity against numerous non-communicable diseases[24&25]. Millets have received the much needed attention after the declaration of the year 2023 as International Year of Millets by Food and Agriculture Organization and the United Nations[17]. Browntop millet is yet to receive the righteous recognition. However, with time browntop millet is gaining prominence due to its huge potential to produce high yield even in resource-poor conditions and having exemplary nutritional values[28].

In the recent past, nanoparticles have captivated worldwide attention due to their unique physical and chemical properties and their widespread use in fields such as health, biology, biotechnology, and chemistry[29&30]. The term “Nano” is derived from the Greek Word “nanos” meaning dwarf, tiny or very small[31&32]. There are different methods for the synthesis of metal nanoparticles (MNPs) that includes physical, chemical, electrochemical, irradiative, photochemical and biological techniques[30,31,32&33]. Although most of the above mentioned methods are effective in producing pure and well-defined nanoparticles, they are either expensive or possibly hazardous to the environment. Among all the methods, biosynthesis of nanoparticles has been proven to be cost-effective, biocompatible, environment friendly, non-toxic, clean and easily scalable for large-scale synthesis. In the green synthesis of MNPs, an array of biomolecules serves as reducing and stabilizing agents[30,31,32,33&34]. Biofabrication of MNPs has been reported by using bacteria, fungi and plant extract. Later being superior to others because firstly, it eliminates the elaborate process of maintaining cell cultures and secondly, the phytochemicals present in plants show greater reduction and stabilization[30&32].

Among several MNPs such as CuNPs, AuNPs, PdNPs, PtNPs, NiNPs and SiNPs, AgNPs are one of the most studied MNPs and have been the focus of interest due to their extraordinary physiochemical features, particularly their enormous surface area, small size, and stability[31,32&35]. The basic principle behind production of AgNPs is the reduction of silver ions to neutral silver atoms[32]. AgNPs have several bioactivities that include properties such as antimicrobial, anticancer, anti-inflammatory, cardio protection and wound healing[31&33].

Plant tissue culture refers to the *in vitro* culture of cells, tissues, organs or whole plant under sterile conditions on a nutrient media of known composition[36&37]. The process involves the use of explants, which are small pieces of plant tissue, to generate new plants or plant parts in an environment that promotes their growth and development, free from pathogens and contaminants[36,37&38]. The nutritional requirements are fulfilled via MS media (Murashige and Skoog) which is the most commonly used nutrient culture media[38].

In traditional cultivation, many plants fail to germinate, flower and produce seeds in particular climatic conditions or have protracted periods of growth and multiplication. Micropropagation ensures a consistent supply of plants with less space and time. Micropropagation is associated with several advantages such as (a) higher multiplication rate (b) provides control over environmental, nutritional and other factors to meet the specific needs of the plant (c) plants are available all year round independent of seasonal or regional variations (d) production of secondary metabolites (e) new and superior genetically altered plants can be developed. Multiple tissue culture techniques are available for micropropagation and plant regeneration; common ones include callus culture, suspension culture, somatic embryogenesis and protoplast cultures[38].

Thus, tissue culture provides a way to carry out experiments even when the experimental plants are not available.

PROBLEMS TO BE INVESTIGATED

The study aims to explore the phytochemicals present in the two minor millets, *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen and to investigate the anticancer and antioxidant potential of green synthesized nanoparticles using seeds, sprouted seeds and *in vitro* grown tissues of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen.

OBJECTIVES OF THE INVESTIGATION

The objectives of the investigation are:

1. Procurement and identification of the sample plants- *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen.
2. Tissue culture from the seeds and other parts of the sample plants.
3. Extract preparation of seeds, sprouted seeds and *in vitro* grown tissues of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen in different organic solvents.
4. Phytochemical analysis of the prepared extracts.
5. Biogenesis and characterization of AgNPs obtained from seeds, sprouted seeds an*d in vitro* grown tissues.
6. Biotesting of synthesized AgNPs for its antioxidant and anticancer activity.

HYPOTHESIS

The treatment of cancer includes several drugs and medications that lead to the rise of various types of side effects. So there is a need to look for drugs that are plant-based and have minimal adverse effects on the body. Plant-based chemical compounds have been utilized to treat human ailments since the beginning of medicine. The therapeutic effects of the plants are dependent on their phytochemical constituents. Based on the function of fundamental metabolic processes, the phytochemical components of plants can be divided into primary and secondary metabolites, later being the key drivers of plants pharmacological actions due to their biosynthetic origin and unique biochemical structure.

Large-scale production of secondary metabolites can be achieved through plant tissue culture, which is an integral part of genetic engineering and is mostly an empirical process. In this way culture tissue, such as callus tissue can be exploited for the production of diverse metabolites. It is hypothesized that, given the right environmental and nutritional conditions, plants that produce a given set of metabolites in their native habitat will inevitably produce those same metabolites in culture, either in smaller or larger quantities.

Nanotechnology is a widely employed targeted drug delivery technique in therapeutic medicine that has recently garnered attention for its unique attributes and applications in cancer treatment. Nanomedicine is the use of nanoparticles for disease prevention, diagnosis,

monitoring, control, and therapy, enhancing medication efficacy through improved bioavailability.

Dr. Khadar Valli popularly known as "Millet man of India", a Krushiratna Awardee has mentioned about the potential of kodo and browntop millet in curing various types of cancers. Kodo millet is effective against cancer of blood, intestine, thyroid, throat, pancreas or liver and browntop millet having positive effects on brain, blood, breast, stomach, intestine and skin cancer**.** However, there is no scientific evidence to support these claims. Therefore, this study is being carried out to ascertain a scientific basis for the use of both the minor millets in the management of cancer.

Taking this into account, the following hypothesis will be tested:

- The synthesized AgNPs using seeds, sprouted seeds and *in vitro* grown tissues of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen shows antioxidant properties.
- The synthesized AgNPs using seeds, sprouted seeds and *in vitro* grown tissues of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen shows anticancer properties.

RESEARCH DESIGN AND METHODOLOGY

1. Tissue culture of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen[39&40].
2. Extract preparation of seeds, sprouted seeds and *in vitro* grown tissues in different organic solvents.
3. Phytochemical analysis of the prepared extracts for the assessment of primary and secondary metabolites such as proteins, carbohydrates, phenols, flavonoids, terpenoids, saponins etc.[41-45].
4. Synthesis of AgNPs from the prepared extracts[29,30&34].
5. Characterization of AgNPs

   It can be done using various techniques such as SEM, TEM, UV-Vis spectroscopy, FTIR, XRD.
6. Antioxidant activity

   For testing the antioxidant properties assays such as DPPH, FRAP or ABTS will be adopted[46&47].
7. Anticancer activity

   This work will be carried out on animal models or different cancer cell lines through MTT assay[48&49].

Sample collection

The seeds and plants of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen were collected from Birsa Agriculture University, Kanke, Ranchi. The plant specimens were certified by CNH, Botanical Survey of India (BSI) Kolkata. The seeds were washed thoroughly with running water followed by rinsing with sterile distilled water and were dried at room temperature for further work.

Tools to be used

- **Chemicals-**

  MS media, 2,4-D, IAA, IBA, BAP, Kinetin, Hexane, Ethanol, Methanol, Ethyl acetate, Distilled water, Mercuric chloride ($HgCl_2$), DMSO, Folin Ciocalteu Reagent, Gallic acid, Aluminum nitrate [$Al(NO_3)_3$], Potassium acetate (CH_3CO_2K), Quercetin, Silver nitrate ($AgNO_3$), DPPH, ABTS etc.

- **Equipments-**

  Autoclave, weighing machine, centrifuge, microwave, pH meter, incubator, rotator shaker, grinder, laminar air flow, SEM, TEM, UV-Vis spectrophotometer, FTIR, XRD, cuvette, petri dish, water bath, culture tubes, culture bottles, filter paper, conical flask, beaker, glass rod, glass plate, funnel, spatula, test tube stand, micropipette etc.

SIGNIFICANCE OF THE STUDY

From antiquity, plants have been an excellent source of bioactive compounds, drugs and cure for various illness. The synthesis of nanoparticles using millets as a biological source has been explored to a very less extent. Also, there has been limited study on the evaluation of biological properties such as antioxidant, antimicrobial, anticancer etc. of *Urochloa ramosa* (L.) T.Q.Nguyen and related green synthesized AgNPs. In contrast, much more studies have been conducted on *Paspalum scrobiculatum* L. related to its phytochemical analysis, antioxidant and anticancer properties. However, no study has been conducted on the synthesis of nanoparticles and their bioevaluation. The chosen minor millets themselves and the fabricated AgNPs have not been explored for the formulation of drugs in treating cancer to much extent.

Hence, the study will be beneficial in the terms of:

- Promotion of the underutilized minor millets.
- The study will provide new insights for the investigation and identification of bioactive compounds naturally present in *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen that may be the cure of different diseases, cancer being the major one. Thus, providing a food source which may be helpful in combating against cancer.
- Tissue culture of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen will help to enhance the production of those beneficial compounds.
- Also the synthesized AgNPs will be examined for their antioxidant and anticancer activity which may aid in targeted action towards cancer.

The tissue culture of plants is based on the principle of “totipotency”. It is the ability of a plant cell to generate a whole new plant on the availability of suitable nutritional and environmental factors. Thus, this study will reduce loss of the existing biodiversity by carrying out studies on the plants regenerated through tissue culture.

IT'S RELEVANCE TO THE PRESENT DAY PROBLEM AND NEED OF THE SOCIETY

Major lifestyle and environmental changes in recent few years have led to the infestation of various diseases in our lives. Conventional treatment through allopathic ways has adverse side effects due to which there is an increasing demand for natural green products among people. As of today, cancer is one of the most common illnesses diagnosed globally, and it's been said to be the main killer in our contemporary culture despite the massive quantity of research and the significant advancements witnessed over the previous ten years. The conventional methods and drugs utilized for the treatment of cancer hold various side effects. So the current study will lead to the affirmation of certain compounds in *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen for developing novel pharmacological molecules. Moreover, it will lead to the discovery of more effective AgNPs for combating cancer that will lead to the formulation of novel drugs for welfare of the society.

CONTRIBUTION TO THE EXISTING KNOWLEDGE

The research conducted on millets so far has mainly focused on major millet varieties such as pearl, sorghum and finger millet. Minor millets have been away from limelight in terms of research. In recent years, the focus has also been put upon some of the minor millets. Thus, the study will put emphasis on the discovery of innate antioxidant and anticancer properties of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen, moreover, the synthesized AgNPs will also help understand their potential to fight cancer in a more efficient way and may lead to the discovery of pharmacological compounds for the formulation of novel drugs. In this way, it will be beneficial for the plant based pharmaceuticals that are looking for the synthesis of new plant based drugs.

FUTURE PERSPECTIVE

The proposed study will deal with the phytochemical analysis, green synthesis of AgNPs from seeds, sprouted seeds and *in vitro* grown tissues of *Paspalum scrobiculatum* L. and *Urochloa ramosa* (L.) T.Q.Nguyen along with the evaluation of antioxidant and anticancer potential for the same. The study will highlight the role and efficiency of obtained AgNPs in tackling cancer to effectively harness the potential of these grains for the treatment of future cancer patients. Further studies can be carried on to evaluate the antimicrobial, anti-inflammatory, antihyperglycemic, hypolipedemic properties of naturally present compounds in both minor millets as well as for green synthesized AgNPs. The present study will also open doors for the exploration of the drug delivery capacity of the green synthesized AgNPs and will assist in the making of novel drugs for the treatment of cancer and other diseases too.

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